REVIEW

Cardiac Cell Repair Therapy: A Clinical Perspective

Bernard J. Gersh, MBChB, DPhil, FRCP; Robert D. Simari, MD, PhD; Atta Behfar, MD, PhD; Carmen M. Terzic, MD, PhD; and Andre Terzic, MD, PhD

From bone marrow transplants 5 decades ago to the most recent stem cell–derived organ transplants, regenerative medicine is increasingly recognized as an emerging core component of modern practice. In cardiovascular medicine, innovation in stem cell biology has created curative solutions for the treatment of both ischemic and nonischemic cardiomyopathy. Multiple cell-based platforms have been developed, harnessing the regenerative potential of various natural and bioengineered sources. Clinical experience from the first 1000 patients (approximately) who have received stem cell therapy worldwide indicates a favorable safety profile with modest improvement in cardiac function and structural remodeling in the setting of acute myocardial infarction or chronic heart failure. Further investigation is required before early adoption and is ongoing. Broader application in practice will require continuous scientific advances to match each patient with the most effective reparative phenotype, while ensuring optimal cell delivery, dosing, and timing of intervention. An interdisciplinary effort across the scientific and clinical community within academia, biotechnology, and government will drive the successful realization of this next generation of therapeutic agents for the "broken" heart.

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GCSF = granulocyte colony–stimulating factor; HSC = hematopoietic stem cell; LVEF = left ventricular ejection fraction; MI = myocardial infarction; MSC = mesenchymal stem cell

It's easy to be excited about stem cell research.
Deborah J. Sweet, PhD[1]
Editor and Executive Editor, Cell Press

This quotation from the editor of the inaugural issue of the journal *Cell Stem Cell* seems apt, given what we already know about the potential for stem cells to play a pivotal therapeutic role in a diverse group of diseases. Some of these treatments, such as bone marrow transplant, have been standards of care for years, but the promise of extending stem cell therapy into other organ systems, including the heart, has understandably generated enthusiasm as well as controversy. Regardless of whether one is a skeptic, an active part of the burgeoning community of stem cell investigators, or an interested clinician, the field is gathering momentum, and it behooves us all to become familiar with the concepts and the lexicon of cell repair therapy as the pace of translational research accelerates.

Accordingly, this review of cardiac cell repair therapy aims to provide a clinical perspective on and outline of the scientific issues underpinning both experimental and clinical studies, highlight the results of randomized controlled clinical trials and the design of future trials, and introduce ethical and philosophical issues of concern.

Cardiac repair can be considered as the outcome of 3 major processes: replacement (tissue transplant), rejuvenation or restoration (activation of resident cardiac stem cells or other stem cells via paracrine or autocrine mechanisms; modulation of apoptosis, inflammation, angiogenesis, or metabolism), and regeneration (progenitor or stem cell engraftment forming differentiated myocytes).[2,3] These different entities may be interlinked in that modulation of myocardial injury may also benefit subsequent therapy directed at myocardial regeneration.[2]

CLINICAL NEED

A compelling clinical need exists for new cardiovascular therapies, including approaches to the protection, restoration, and regeneration of cells. Despite the improvements in acute care and the impact of primary and secondary prevention, coronary artery disease remains the leading cause of death in the United States, and the decline in mortality rates that began in the 1960s slowed somewhat in the 1990s.[4-7] Approximately 1 million myocardial infarctions (MIs) occur per year in the United States, with a 25% mortality rate at 3 years; approximately 5 million patients have heart failure, with a 20% annual mortality rate. Moreover, cardiac transplant will not fill the need given that donors are lacking and xenotransplantation remains experimental. To place these data in a broader context, the *global* nature of the epidemic of cardiovascular disease should be taken into account.[8,9]

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SCIENTIFIC OVERVIEW

As a concept, cell therapy is intuitively appealing.[2,10-15] For conditions characterized by myocyte loss, ie, MI and heart failure, the postulate is that nonviable myocardium may be

From the Division of Cardiovascular Diseases (B.J.G., R.D.S., A.B., A.T.) and Department of Physical Medicine and Rehabilitation (C.M.T.), Mayo Clinic, Rochester, MN.

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Individual reprints of this article are not available. Address correspondence to Bernard J. Gersh, MBChB, DPhil, Division of Cardiovascular Diseases, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (gersh.bernard@mayo.edu).

regenerated or repaired by delivery of stem or progenitor cells from a variety of sources, including the heart itself. Traditionally, the cardiomyocyte has been considered terminally differentiated, with the response to injury characterized by hypertrophy and not hyperplasia.[13] Recent evidence raises the possibility that a natural system of myocyte repair exists; however, less than 50% of cardiomyocytes are exchanged during a normal life span, and the system appears to be inadequate to the magnitude of an ischemic or heart failure insult.[16] Nonetheless, the capacity of the adult human heart to generate myocytes suggests that it is rational to work toward the development of therapeutic strategies aimed at stimulating this process.[10,16]

Although the concept of myocyte repair is straightforward in theory, realizing the potential of therapeutic strategies based on this concept is extraordinarily complex, and the magnitude of this task has been highlighted recently.[2,12,15,17-23] The "myocyte-deficit" in infarction-induced heart failure, which results in an approximately 25% loss of the left ventricle, is on the order of 1 billion myocytes.[20] For therapy to be successful, not only must regeneration occur on a large scale, but contraction needs to be synchronous and electromechanically coupled with vasculogenesis to ensure cell nourishment.[2,12]

STEM CELLS: ADVANTAGES AND DISADVANTAGES OF DIFFERENT CELL TYPES

In preclinical and clinical studies, a variety of cells have been considered as candidates for cell repair therapy (Figure 1). Cells differ markedly in regard to their site of origin as well as to their anatomy and function, as characterized by surface markers, transcription factors, and expressed proteins. They also differ in their ability to form 1 or more differentiated cell types (Figure 1).

Embryonic Stem Cells

The embryonic stem cell, derived from the inner mass of the developing embryo during the blastocyst stage, has the greatest potential for organ regeneration and is the prototypical stem cell.[15,24-27] Embryonic stem cells can evolve into a variety of cell types and tissues, including cardiomyocytes; however, their innate aptitude for pluripotent proliferation also presents an increased risk of teratoma.[28-30] In animal models of experimental MI and nonischemic cardiomyopathy, embryonic stem cell transplant has resulted in a remarkable improvement in cardiac function and structure, and the cells appear to be electrically integrated.[31-34] A potential challenge for clinical translation is the immunological mismatch of embryonic stem cells due to their allogeneic origin.[35] Furthermore, the methods by which embryonic stem cells are derived have raised social and ethical concerns, hampering the discovery process for this phenotype both in the preclinical and clinical arena.[21,36]

The concept of *guided cardiopoiesis*, in which mimicry of the natural embryonic cardiogenic milieu is used to derive a cardiac progenitor population, is a major focus of stem cell research aimed at ensuring predifferentiation before application.[31,37-39] The molecular underpinnings of this process are identified by genomic and proteomic characterization of natural cardioinductive signals, leading to the establishment of a recombinant approach to achieve "cardiopoietic guidance."[40,41] In addition to signaling molecules, this approach exploits synergism between growth and trophic factors to replicate the impact of the endodermal secretome to focus the unguided plasticity of pluripotent stem cells toward a specific myocardial pathway, nullifying the propensity for uncontrolled growth.[39] This process of "guided development" is currently being driven into the clinical arena, with similar approaches applied to mesenchymal stem cells (MSCs) obtained from the bone marrow to enhance the latent cardiogenic potential of adult cell phenotypes.[39] An alternative strategy generates purified human embryonic stem cell–derived cardiomyocytes, demonstrating a robust postimplantation survival after stimulation with procardiogenic and prosurvival factors.[37] Moreover, biomarker-based prediction of emergent cardiogenic specification within a stem cell pool enables targeted selection of cardiopoietic lineage.[42] In addition to replacing diseased tissues, embryonic stem cells can overcome metabolic deficiencies and restore disconnected cellular interactions. Collectively, these beneficial effects may prove to be a critical component of the regenerative capacity in models of human disease, providing future avenues for regenerative medicine.

Adult Stem Cells: Bone Marrow–Derived Cells

In contrast to pluripotent embryonic stem cells, adult stem cells display more limited differentiation capacity. The bone marrow exemplifies a typical adult stem cell source, containing different cell populations that have the potential to migrate and transdifferentiate into cells of diverse phenotypes. The extent to which these cells could differentiate into cardiac myocytes is uncertain, and findings of animal studies have not been replicated consistently.[43-46] The 2 major subsets of bone marrow cells, hematopoietic stem cells (HSCs) and MSCs, can be further categorized into subpopulations and stratified by the expression of cell surface markers. Bone marrow cells may be isolated by direct marrow aspiration or can be obtained from the peripheral circulation after cytokine mobilization.

Hematopoietic Stem Cells. Commonly identified by the expression of $CD34^+$ and CD133 cell surface antigens, HSCs have been studied extensively and used clinically for bone marrow transplant for a variety of hematologic disorders. Cells capable of assuming an endothelial phe-

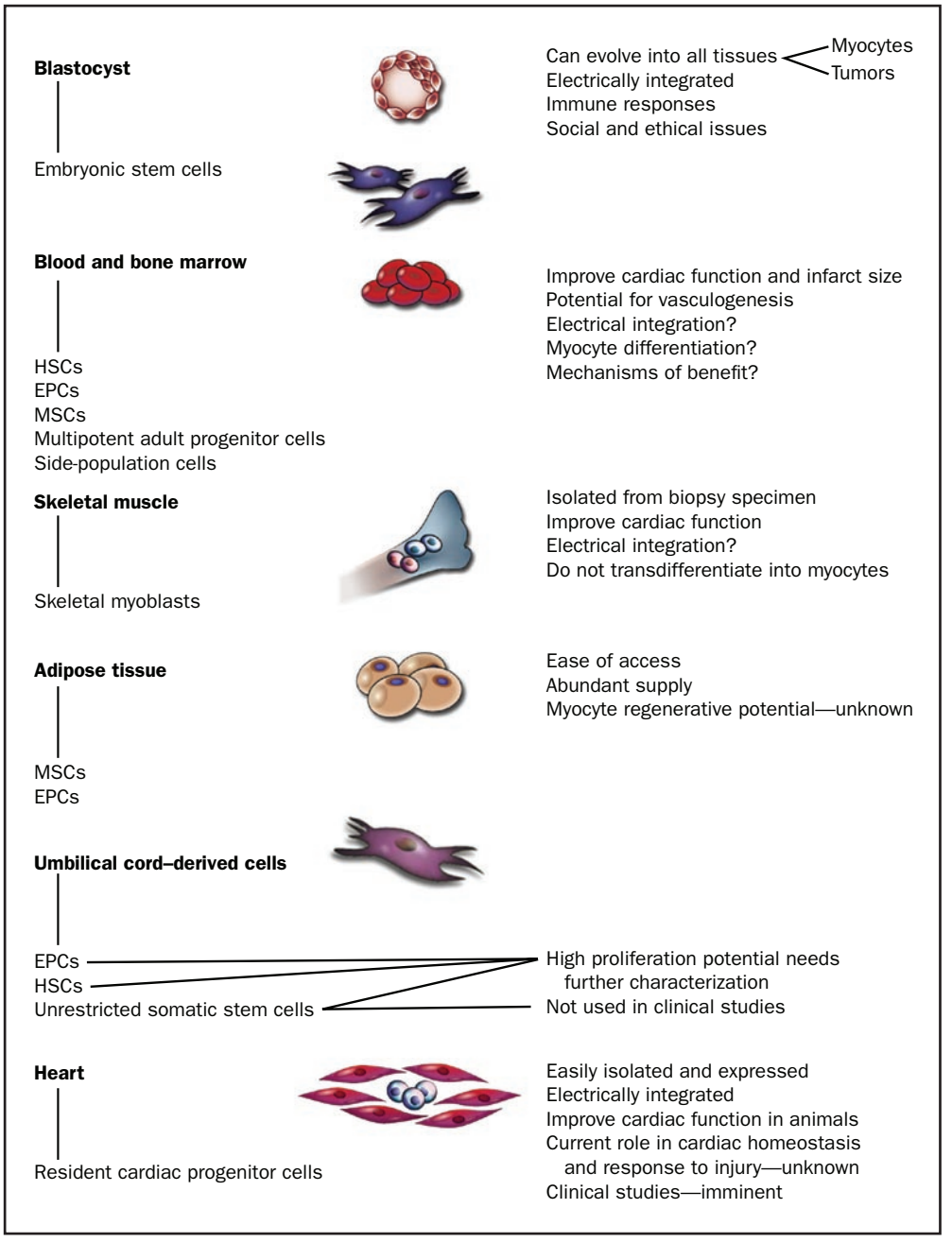

FIGURE 1. Advantages and disadvantages of different cell types isolated from different sources used both clinically and experimentally. EPC = endothelial progenitor cell; HSC = hematopoietic stem cell; MSC = mesenchymal stem cell.

notype have been identified in the blood and bone marrow. Endothelial progenitor cells, a heterogenous population of cells that also reside predominantly in the bone marrow, likely promote neovascularization by secreting proangiogenic growth factors and by stimulating re-endothelialization; both functions could contribute to vascular homeostasis and perhaps myogenesis.[47]

Other cells located in the bone marrow (but not exclusively so) include MSCs (discussed in the following section), multipotent adult progenitor cells, and side-population cells, which are defined by molecular mechanisms allowing exclusion of Hoechst dye. The potential regenerative role of these cell types is based on findings in experimental animal models, not on clinical experience.[48-51]

It remains to be determined whether bone marrow cells or cell lines from other sources will be favored for myocyte formation in the future. Unless the efficacy of existing bone marrow cell preparations is substantially enhanced, attention will likely shift to other sources of cells.

Mesenchymal Stem Cells. A population of cells present in adult tissues including the bone marrow and adipose tissues,[52] MSCs are characterized by an absence of HSC markers. They can be isolated and expanded easily and, as experimental studies have shown, can transdifferentiate into functional cardiomyocytes and a variety of other cells, resulting in an improvement in left ventricular function and remodeling.[53-56] They can also modulate immune responses.[57] The magnitude of transdifferentiation is currently under investigation, but accumulating evidence supports the active engraftment and differentiation of transplanted human MSCs within the healing myocardium in sheep.[58] This finding has been corroborated in vitro, with cardiogenic MSC guidance demonstrating a capacity for sarcomerogenesis and electromechanical coupling.[59] Noninvasive multimodality imaging indicates that therapy after MI with allogeneic MSCs promotes active cardiac regeneration in vivo.[60]

Adipose tissue derived from the embryonic mesenchyme contains MSCs and endothelial progenitor cells as well as adipose cells. Experimental data suggest that adipose tissue–derived cells may transdifferentiate into cells with the characteristics of cardiomyocytes and perhaps blood vessels, or at least neovascular tissue.[61,62] Adipose cells are attractive because access to them is easy and they are not in short supply in most societies. Nonetheless, additional characterization and demonstration of efficacy in animal models are needed before a clinical role for these cells can be established.[63]

Fetal and Umbilical Cord Blood Cells

Because of their prenatal origin, fetal and umbilical cord blood cells may possess greater plasticity than adult cells; however, to date, evidence of pluripotency after in vitro expansion is lacking. Human umbilical cord blood contains a number of progenitor cell populations, including HSCs and MSCs, in addition to a population of unrestricted somatic stem cells, which have been shown to have proliferative potential, but animal studies have been conflicting in regard to improvements in left ventricular function.[64] These cells have not yet been investigated in a clinical setting.

Resident Cardiac Stem Cells

Several clusters of surviving resident cardiac stem cells or progenitor cells have been identified in the hearts of humans and other mammalian species.[22-24,65,66] Lineage-tracing experiments are needed to determine precisely the extracardiac vs intracardiac origin of these cells. Their actual role in myocardial homeostasis during life is unknown; although they have a high proliferative potential, it is inadequate to compensate for extensive injury, as occurs with acute MI.[67]

Cardiospheres, which are spherical clusters of cells that can be obtained with a cardiac biopsy, are plated and grown in culture to yield cardiosphere-derived cells in addition to other populations of resident cardiac progenitors, including c-kit$^+$ cells.[22] A recent proof-of-concept study showed that cardiospheres obtained from the myocardium by endomyocardial biopsy could be isolated, cultured, and expanded to provide a potentially useful source of autologous cardiac stem cells.[66] The findings of these and other studies using c-kit$^+$ cardiac stem cells have demonstrated feasibility and documented the benefits on left ventricular function, remodeling, and infarct size in animal models; however, these benefits are inconsistent.[24,68,69] In response to acute infarction, cells expressing c-kit$^+$ and other cardiogenic markers were present in the myocardium, suggesting a role in cardiac repair and also in early cardiac development because the population of these cells is markedly increased in the postnatal (1-2 weeks after birth) vs the adult mouse heart.[70] A key, but as yet unanswered, question is whether a specific resident cardiac progenitor cell would be more effective than a mixture of cells, including c-kit$^+$ cells,[66] SCA (stem cell antigen)-1$^+$ cells,[69] side-population cells,[70] cardiospheres, and cells expressing the transcription factor islet 1.[71] Clinical trials are in the planning phase.

Skeletal Myoblasts

Skeletal myoblasts were the first cells to be injected into the ischemic myocardium as part of a cell-based strategy.[72] Despite reported improvements in left ventricular function and volumes, possibly via a mechanical or scaffolding effect, little evidence shows that these cells can transdifferentiate into cardiomyocytes.[73-75] Moreover, the improvements in left ventricular function do not appear to be sustained, and the cells are not electrically integrated and, as such, may predispose to arrhythmias.[76] The 1-year follow-up of a recent small randomized controlled trial in which skeletal myoblasts were delivered via a 3-dimensional guided catheter system was favorable in regard to left ventricular function, symptom relief, and quality of life.[77] Nonetheless, it would appear that enthusiasm for this approach is waning; however, considerations for modified or preselected products have been formulated, and a "second generation" of skeletal myoblasts modified by cell enhancement techniques has been hypothesized.[74,78]

Induced Pluripotent Stem Cells

In an important recent breakthrough, populations of cells with characteristics reminiscent of embryonic stem cells were generated from somatic tissue, such as adult fibro-

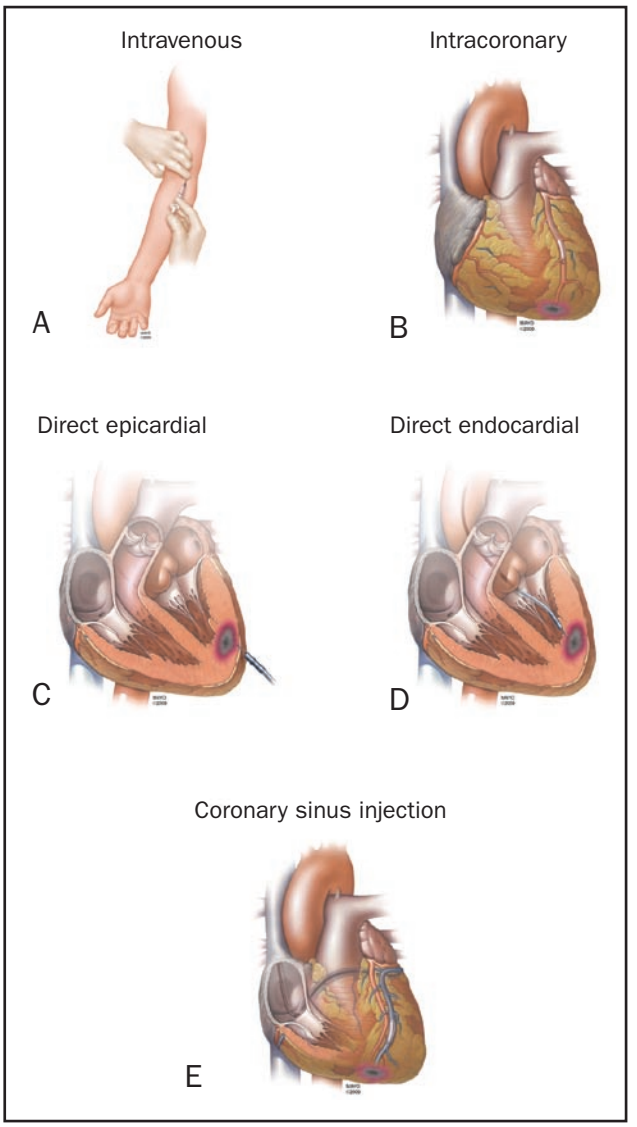

FIGURE 2. Methods of cell delivery for cardiac implantation. A, intravenous administration; B, intracoronary infusion using a balloon catheter after restoration of arterial patency; C, transepicardial injection via thoracotomy into the border zone of the infarct; D, transendocardial approach using electromechanical voltage mapping to define tissue viability; and E, intravenous injection into the coronary veins via the coronary sinus, enabling cell delivery into myocardial areas subserved by occluded coronary vessels.

blasts, through nuclear reprogramming using ectopic expression of genes related to pluripotency.[79-84] This revolutionary approach provides an alternative source from which to generate cell lines with cardiogenic potential without the use of eggs or embryos. In addition, this strategy could be used to develop patient-specific stem cells, which could be a unique resource in studying genetic mechanisms of disease development, drug actions, and regenerative biology. A recent study suggested that the use of fat cells liposuctioned from middle-aged patients markedly increased the efficiency of pluripotent stem cell induction.[85] To improve safety, the technique has been refined recently to incorporate virus-free approaches for gene delivery.[86] A recent report showed generation of human-induced pluripotent stem cells by direct delivery of reprogramming proteins that are free of DNA vectors.[87] The clinical implications of these revolutionary conceptual developments remain to be determined, but the potential is indeed exciting, given the recent demonstration that human-induced pluripotent stem cells or fibroblasts transduced with human stemness factors can differentiate into functional myocytes.[88,89]

ROUTES AND METHODS OF CELL DELIVERY

The strategy of cell therapy is to repair injured tissue through delivery of an adequate cell dose to an area of interest. Achieving this goal requires a conducive microenvironment for cell survival, retention, and/or homing, among other factors. Currently available routes of administration include intravenous,[90] intracoronary,[91] transmyocardial (by direct epicardial injection),[92] catheter-based transendocardial injection using electromechanical voltage mapping,[93,94] and a recently implemented approach of transvenous injection into coronary veins[74,95,96] (Figure 2). Intrapericardial delivery is under investigation. No single strategy has emerged as the preferred technique. The timing of administration in relationship to the onset of disease (eg, MI) is also key, as is the underlying disease substrate (ischemic vs nonischemic). In the case of ischemic disease, no consensus yet exists as to whether cells should be placed in the area of the scar, the microvascular dysfunction, or the border zone; appropriate placement would be an important determinant of the optimal strategy in specific subgroups. The advantages and disadvantages of the different strategies are comprehensively discussed in a recent review.[97] In general, the efficiency of delivery and retention is lower than hoped, and retention and survival of cells at sites of delivery are limited.

PRECLINICAL AND CLINICAL STUDIES: IMPLICATIONS AND MECHANISMS OF BENEFITS

The striking feature of multiple animal, preclinical, and early clinical studies, excluding the randomized controlled trials, is the near universality of benefit, in terms of improvement in ejection fraction, ventricular volumes, infarct size, and myocardial perfusion (Figure 3). "Everything seems to work," and these benefits have been noted for all cell types, for both allogeneic and autologous cells, and for autologous cells of both intracardiac and extracardiac origin.[98] Moreover, these effects appear to be independent of the timing after MI, etiology (ischemic vs nonischemic), the method and site of deliv-

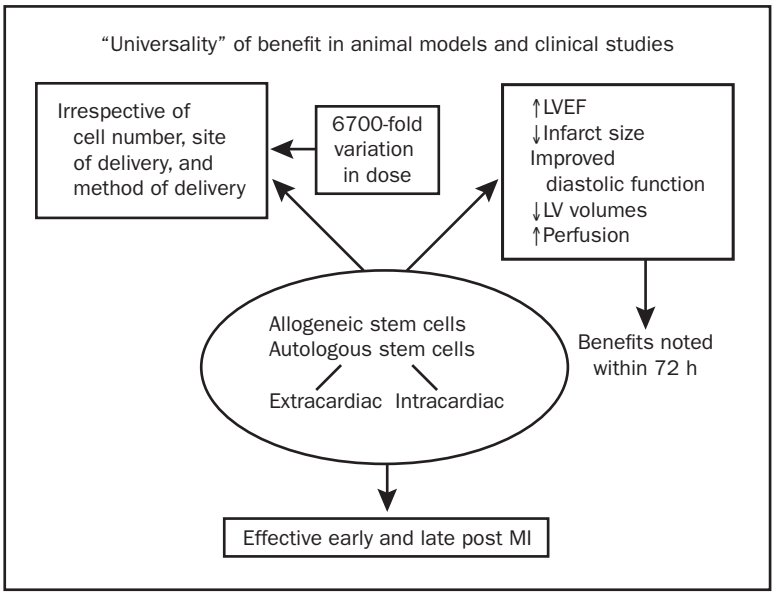

FIGURE 3. Concept of the universality of benefit noted in animal and clinical studies, with the exception of some recent randomized trials that have reported discordant results. The 6700-fold variation in dose among different studies is obtained from reference 3. LV = left ventricular; LVEF = LV ejection fraction; MI = myocardial infarction. From *Indian Heart J*.[98]

ery, and the number of cells administered. Indeed, Murry et al[3,20] have pointed out that doses in reported studies range by as much as 6700 fold. Another striking feature is the documentation of left ventricular functional improvement within 72 hours—far earlier than would be expected for cell regeneration of any meaningful extent.[99]

Whether stem cells from the bone marrow, and in particular HSCs, can transdifferentiate into cardiomyocytes has been an area of considerable debate during the past decade[2,3,10,12,13,15,19,20,100,101] (Figure 4). Trial results have shifted opinion somewhat; it is currently generally recognized that injection of HSCs does not lead to cardiomyocyte differentiation, at least not in numbers that would be physiologically and clinically meaningful, and that cell retention after the first few days is minimal (Figure 4). A recent genetic proof-of-concept study, however, showed that transplanted bone marrow cells can induce cardiac gene expression, although the number of these cells that acquire a cardiac phenotype is low.[102] The concept of *cell fusion* (ie, transplanted cells fuse with other cells, resulting in a hybrid cell progenitor with differentiated cell markers) has been demonstrated in vitro, but its clinical relevance is disputed and generally considered to be small in regard to cardiac regeneration.

A focus of intense investigation in a number of laboratories worldwide is whether other types of cells, including MSCs, embryoid body–derived cells, and resident cardiac progenitors, as well as genetically programmed mature adult cells, can transdifferentiate into cardiomyocytes. A key component of these efforts will be an elucidation of the complex signaling mechanisms that control the process of "guided development" into myocytes, thus allowing the population to be enriched in regard to growth retention and survival.[31,37-39,43-45,47] This is potentially equally instructive in regard to understanding why pluripotent stem cells can form teratomas.

Nonetheless, for the present, we are faced with a paradox: the overwhelming conclusion from multiple sources is that cell transplant translates into a range of beneficial responses, but these occur in an environment characterized by a lack of clinically or pathophysiologically relevant cell transdifferentiation, retention, and survival. Although we should be mindful of these caveats, evidence of efficacy should not be dismissed just because mechanisms are not understood. Although a vigorous regenerative capacity has not been demonstrated with current cell populations, that does not preclude the potential for enhancement of endogenous repair capabilities through a variety of other mechanisms, nor does it mean that ongoing efforts to enhance regeneration are doomed to failure.[101]

The injection of stem cells into the wall of the myocardium may alter ventricular geometry and improve remodeling via a scaffolding effect (Figure 4). In an infarct model in rats, Dai et al[103] showed that the injection of collagen into the infarcted wall thickened the scar and resulted in an improvement in stroke volume, ejection fraction, and paradoxical systolic bulging. Whether similar effects occur in humans is unknown.

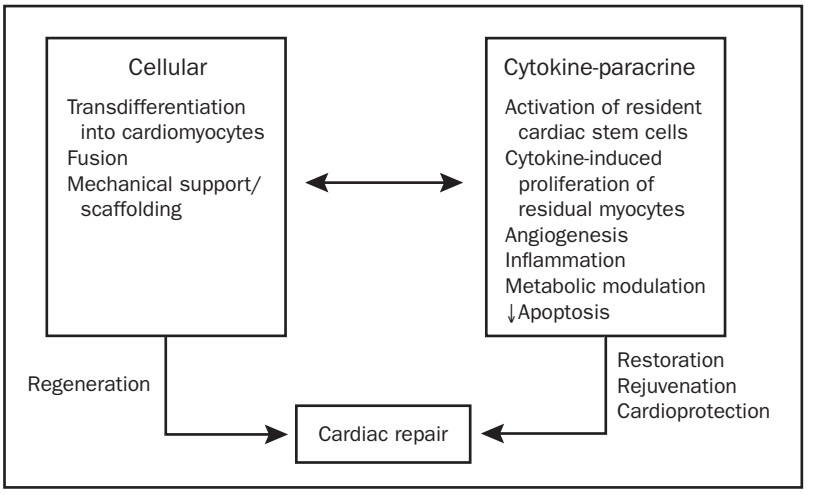

FIGURE 4. Schematic illustrating potentially beneficial mechanisms underlying cell repair or cell regenerative therapy. Prevailing concepts have shifted away from direct cellular effects resulting in transdifferentiation into cardiomyocytes and toward the cytokine-paracrine hypothesis. The underlying rationale is that transplant of cells or factors developed in culture media enhances angiogenesis, curbs inflammation, improves metabolic modulation, and reduces apoptosis, resulting in increased reparative and cardioprotective responses as opposed to regeneration per se.
Adapted from *Indian Heart J*.[98]

Currently, the prevailing concept of stem cell efficacy has shifted toward the cytokine-paracrine hypothesis (Figure 4). Animal studies have shown that the injection of the conditioning medium in which MSCs were cultured results in improvements in left ventricular function and reduced apoptosis.[99,104] In a subsequent article, SFRP2 (secreted frizzled-related protein II), which modulates the Wnt (wingless-type MMTV integration site family) signaling system and the expression of antiapoptotic genes, was shown to be the key factor released by AKT-1(v-akt murine thymoma viral oncogene homolog 1)–enriched MSCs.[105] Recent experimental data have shown that interleukin 10 from transplanted bone marrow mononuclear cells may contribute substantially to cardiac protection after MI.[106] Other cytokines and growth factors from transplanted progenitor cells that may exert important paracrine effects include vascular endothelial growth factor, stromal cell–derived factor, angiopoietin 1, hepatocyte growth factor, insulinlike growth factor 1, and periostin, among others.[44,107-112] Many cytokine and paracrine factors that favorably affect angiogenesis, inflammation, cytoprotection, metabolic modulation, and apoptosis will likely be identified in the future. What is conceivable but perhaps less likely is that these paracrine factors activate or recruit resident cardiac stem cells or induce the proliferation of residual myocytes. Irrespective of the precise mechanisms involved, the intriguing possibility exists that cell repair therapy exerts major beneficial effects, independent of any direct effect of cells on myocyte regeneration. However, such benefits need to be confirmed by further research.

CLINICAL TRIALS: POTENTIAL AND PITFALLS

Clinical Trials–Are They Premature?

In 2001, the first reports demonstrating possible myocardial regeneration in animals using bone marrow–derived cells were followed within approximately 6 months by the first of multiple clinical trials. Given the lack of our current understanding of the mechanisms underlying the differentiation of stem cells into myocardial cells and the mechanisms of apparent benefit, a valid issue is whether clinical trials are premature. Indeed, a moratorium on new clinical trials has been proposed until a number of important questions have been answered in the experimental setting.[113]

One can, however, mount a strong argument for the continuation of carefully designed and focused trials.[114] An unmet clinical need, supportive preclinical data, and a promising early clinical experience in regard to both safety and efficacy provide a powerful incentive to continue along the pathway of carefully designed trials. This has prompted the National Heart, Lung, and Blood Institute to establish the Cardiovascular Cell Therapy Research Network to parallel efforts from other sources in other countries.[114-116] Trials provide answers to preconceived hypotheses, but they also generate a whole new range of questions. Many precedents illustrate the contributions of clinical trials to our understanding of the action of drugs, including aspirin, statins, angiotensin-converting enzyme inhibitors, and aldosterone antagonists.[116] Moreover, preclinical studies in animals will not answer the many complex issues regarding the timing

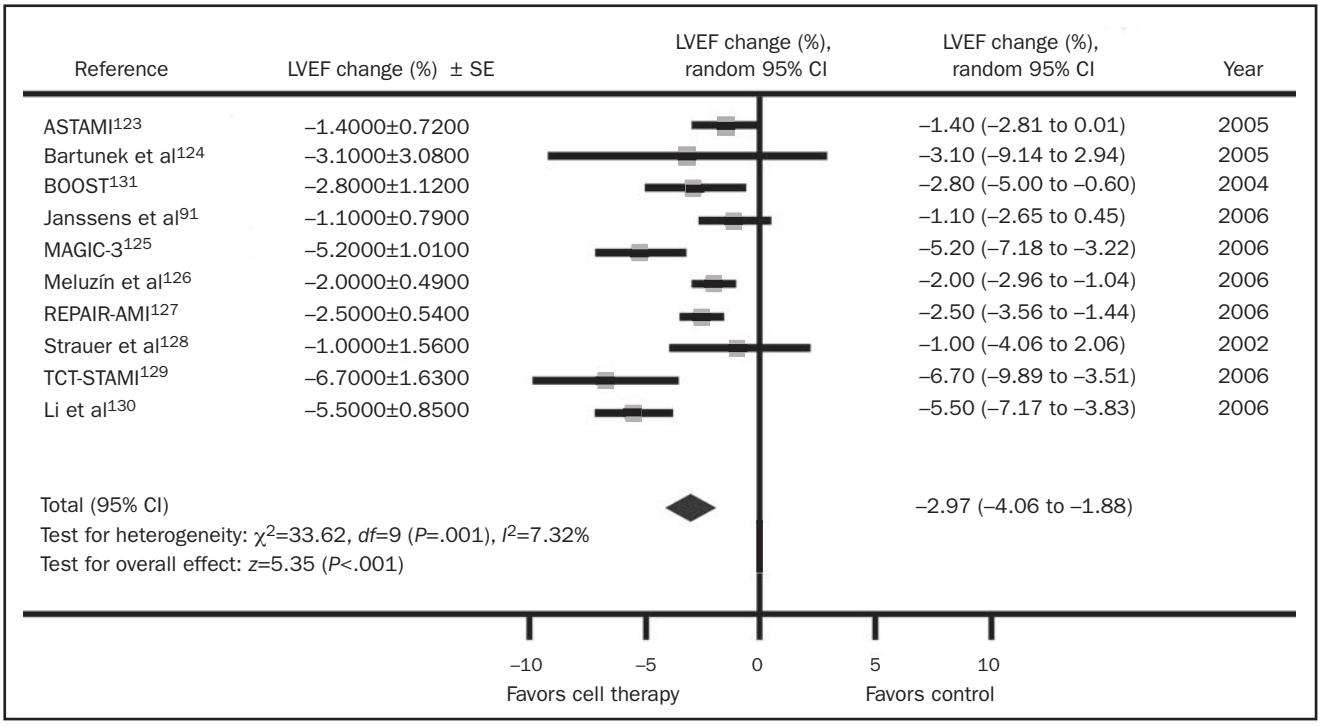

FIGURE 5. Meta-analysis of 10 randomized controlled trials enrolling 698 participants comparing patients diagnosed as having acute myocardial infarction who were treated with autologous stem-progenitor cells with those who were not. A random-effects model was used, and marked heterogeneity was observed between trials. Nonetheless, a consistent pattern suggests that bone marrow stem cell treatment improves short-term left ventricular ejection fraction (LVEF), with similar trends for left ventricular end-systolic and end-diastolic volumes, infarct size, and regional cardiac wall motion (not shown). A positive correlation was also found between cell dose infused and the effect on LVEF measured with magnetic resonance imaging. Conclusions could not be drawn on clinical outcomes such as mortality because of insufficient events. ASTAMI = Autologous Stem Cell Transplantation in Acute Myocardial Infarction; BOOST = Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration; CI = confidence interval; MAGIC = Myoblast Autologous Grafting in Ischemic Cardiomyopathy; REPAIR-AMI = Intracoronary Progenitor Cells in Acute Myocardial Infarction; TCT-STAMI = Emergent Transcatheter Transplantation of Stem Cells for Treatment of Acute Myocardial Infarction. From *J Am Coll Cardiol*,[121] with permission.

and methods of cell delivery. We think that clinical trials should continue but that they should be focused, mechanistic, and based on a close basic and translational collaboration; in this way, the key questions, including safety issues and mechanisms, can be addressed. An understanding of the current limitations of our knowledge base is necessary but should not limit efforts to harness what we have learned to date in an attempt to understand the clinical issues.[116]

Results of Clinical Trials

The promising results of preclinical and early pilot studies launched a wave of clinical trials characterized by methodological heterogeneity, a lack of standardization, and a lack of uniformity in regard to the primary measure of interest and methods of measurement.[12] Although most trials have demonstrated a benefit, several recent trials have yielded disparate results.[117,118] Four meta-analyses of adult bone marrow cells in the setting of acute MI,[119-122] incorporating 5, 10, 13, and 18 trials, respectively, help to place the results of individual trials into perspective. Overall, the results of these placebo-controlled trials are promising in that they demonstrate feasibility, safety, and a modest benefit on left ventricular ejection fraction (LVEF) (an increase of approximately 3%); a reduction in ventricular volumes; a reduction in infarct or lesion size, ranging from 3.5% to 5.6%; and improved regional function.[121] The change in LVEF across a number of these studies is shown in Figure 5.[91,121,123-131] Moreover, the injected cell volumes appear to correlate with the change in LVEF, suggesting a possible dose-response relationship. The greatest improvement is in areas with the greatest damage or extent of scarring.[91] Some trials suggest that myocardial perfusion and coronary flow transmural reserve are improved by bone marrow cell transplant, as is diastolic function.[132,133] In contrast, the BOOST (Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration) trial showed that improvement may be short-lived, raising the possibility that an additional infusion after an unspecified time period may provide added benefit.[131,134]

Somewhat surprisingly, the meta-analyses showed a trend toward a reduction in recurrent MI, and in the RE-

PAIR-AMI (Intracoronary Progenitor Cells in Acute Myocardial Infarction) trial of 204 patients (the largest to date), significant reductions in mortality, rehospitalization for heart failure, and repeated revascularization were reported.[135] These trials were not powered to address effects on "hard" clinical end points, and many precedents exist for positive clinical findings in small trials not standing up to the rigorous scrutiny of large, adequately powered trials.[136] Moreover, the overall benefit demonstrated in the meta-analyses in regard to ventricular function needs to be tempered by the results of the 3 other trials,[117,118,134] which demonstrated either no benefit or an initial benefit that was not sustained beyond 6 months. It has been suggested, and it is certainly logical, that differences in cell isolation and storage protocols could have had an impact on the functional capacity of cells used in the REPAIR-AMI1 and ASTAMI (Autologous Stem Cell Transplantation in Acute Myocardial Infarction) trials and could account for the discordant results; these methodological issues remain a focus of ongoing investigation.[117,137,138]

Perceptions of the results of this first phase of clinical trials are mixed. On the one hand, most transplanted cells disappear from the myocardium within 1 week, and the lack of any concrete evidence of myocyte regeneration is a source of frustration. On the other hand, this also provides an opportunity for further investigating the basic mechanisms of benefit in addition to developing strategies that would enhance cell survival and retention. The trend toward benefit in regard to left ventricular function, perfusion, and infarct size in addition to apparent safety is reassuring and has generated new questions for the next phase of clinical trials. Perhaps unbridled optimism has given way to a sense of reality and an appreciation of the multiple issues that must be addressed both at the bench and at the bedside in a close collaborative environment.

Fewer randomized trials of transplants of blood- or bone marrow–derived stem cells have been performed in the setting of chronic coronary artery disease and chronic heart failure.[139-142] Nonetheless, the results are similar to those in patients with acute MI, showing an improvement in regional and global left ventricular function, perfusion, and relief of angina pectoris (most encouraging in 2 studies).[143,144]

Unresolved Clinical Questions

For the present, the clinical role of stem cell transplant should be confined to a research or clinical trial setting. Prior trials and preclinical studies have provided a roadmap; however, a host of unanswered questions remain to guide future studies. These include whether cell therapy exerts its beneficial effects through differentiation into myocytes or blood vessels or through cytokine-paracrine mechanisms that modulate metabolism, inotropism, apoptosis, and inflammation (Figure 6).

Key clinical issues include patient selection criteria and the impact of comorbid conditions such as advanced age, diabetes, smoking, or hypertension on cell functionality, the timing of administration in relationship to the onset of MI, and potential benefits in nonischemic cardiac disease.[145] Other critical issues include which cells should be transplanted and in what numbers; the methods and optimal sites of delivery; cell isolation and storage procedures; augmentation of cell homing, retention, and survival; and safety concerns. Given the heterogeneity of cell populations under study, head-to-head comparisons between cells and mixtures of cells under controlled conditions would appear to be a priority (Figure 6).

Currently, 25 new clinical trials are in progress in the United States, and a similar number are ongoing in Europe[146]; however, no agreement has been reached regarding the standardization of methods, especially cell harvest, isolation, and preparation.[147] Welt and Losordo[148] have raised the issue of the pharmacokinetics/pharmacodynamics of cell therapy and have drawn attention to the dilemma of whether a dosage refers to the number of cells delivered, the number of cells initially retained within tissue, or the number of cells eventually incorporated into myocardial tissue. All 3 questions may be clinically relevant and highlight the need for new methods of assessment.

Several clinical observational studies raise a key question: why is repair inadequate in adult hearts, despite evidence of endogenous or spontaneous progenitor cell mobilization? In a study of survivors of MI, Leone et al[149] reported a correlation between the concentration of spontaneously mobilized $CD34^+$ cells in the blood and subsequent improvements in left ventricular contraction and remodeling. The greater the degree of cell mobilization, the greater the benefit on left ventricular function. A recent small study of patients with acute MI demonstrated the spontaneous mobilization of very small embryonic stem cells expressing markers of pluripotency.[150] These cells, which are endogenous, autologous, and unmodified, and which have markers suggesting pluripotency, could potentially represent a type of cell for use in cardiac repair and, as such, could bridge the gap between adult and embryonic stem cell phenotypes.[151] The challenges and barriers to cardiac regeneration are formidable, and it is unsurprising that the regenerative capacity of transplanted cells is limited,[114,119] given the hostile microenvironment after MI, characterized by inflammation, fibrosis, and inadequate angiogenesis. Overcoming these hurdles will probably require a multifaceted approach based on augmentation of intrinsic cell function and survival and modification of the milieu into which cells are transplanted.

Are sufficient numbers of cells recruited and mobilized? Is the time period too short? Does microvascular injury im-

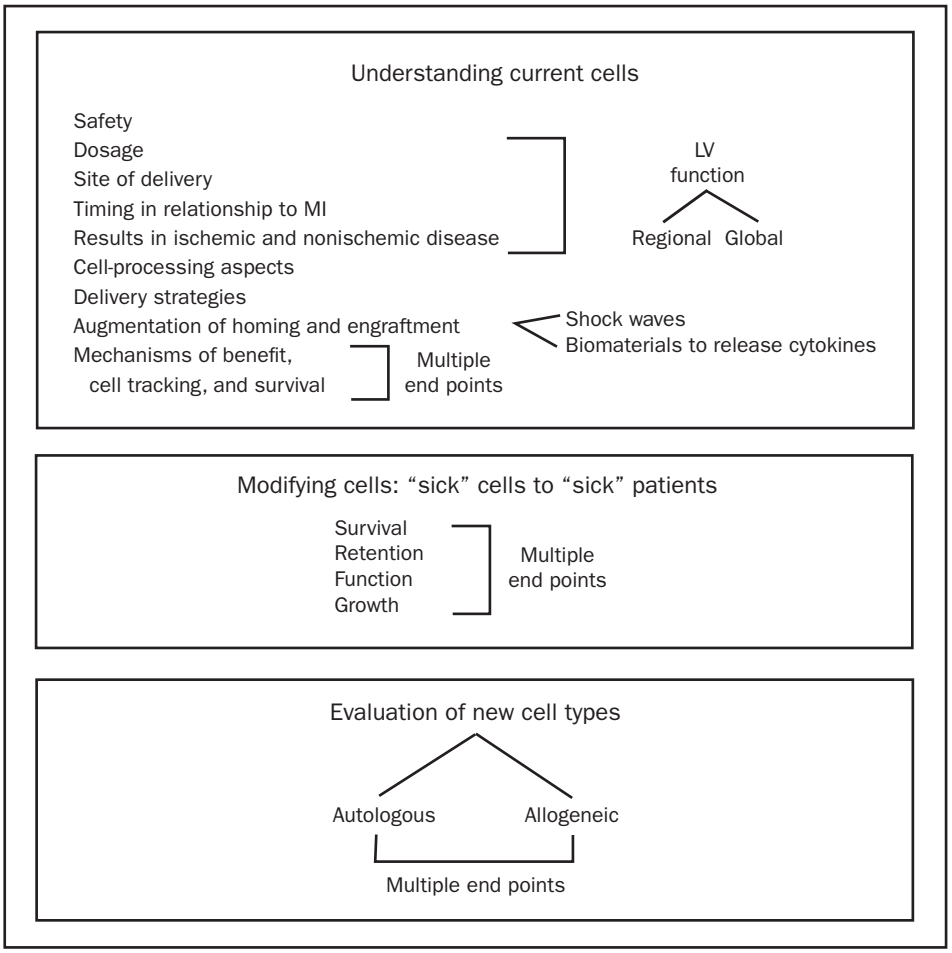

FIGURE 6. The overall objective of ongoing and future clinical trials is to obtain a better understanding of current cells before moving on to the modification of cells and evaluation of new cell types. LV = left ventricular; MI = myocardial infarction.

pede cell penetration into the infarct zone? Is the microenvironment in the region of the infarct hostile to cell homing and viability, and will transplanted cells interact adversely with host myocardial and inflammatory cells? Another area of interest relates to the decline in the number and functionality of endothelial progenitor cells in association with comorbid conditions and whether such "sick" cells can be functionally rejuvenated before transplant. Experimental studies of cell-enhancement strategies have identified a number of novel and intriguing options for improving survival, retention, integration, and homing.[152] These include the transduction of cells with prosurvival genes (eg, the protein kinase Akt, telomerase reverse transcriptase [the active subunit of telomerase], vascular endothelial growth factor, and integrin-linked kinase) and the pretreatment of cells with small molecules (eg, statins, P38 inhibitors, and endothelial nitric oxide synthase [NOS3]) so as to activate the Akt/NOS3 pathway.[153,154] Other approaches focus on pretreatment of the target tissues to enhance active cell recruitment, survival, and retention because stem cell engraftment and survival are highly sensitive to the local cellular environment.[155] These potential strategies include modification of the target region by low-energy shock waves and the introduction of growth factors (eg, stromal cell–derived factor).[152] The enhancement of stem cell therapy through genetic modification of stem cells before transplant provides an alternative innovative approach.[156,157] A composite strategy based on cell proliferation and suppression of local inhibitors has been successful in regard to axonal regeneration in mice with total limb paralysis and conceivably could be used in cardiac regeneration.[158]

An advanced understanding of these patient-related issues in regard to the outcomes and design of clinical trials is essential. The underlying primary diagnosis and disease substrate (ie, acute MI, ischemic vs nonischemic cardiomyopathy, or primary vascular disease) along with the timing of cell administration relative to the onset of infarction

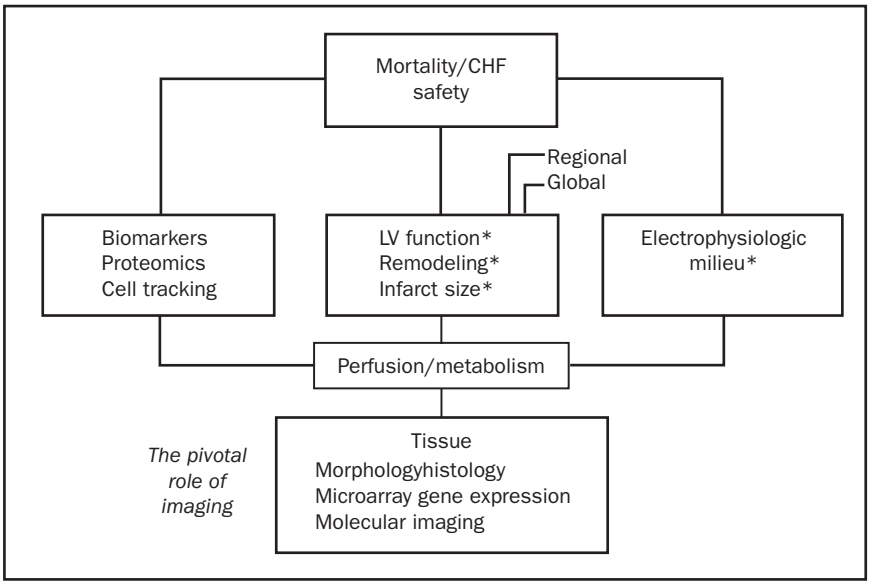

FIGURE 7. This illustrates the hierarchical classification of end points in clinical trials. CHF = chronic heart failure; LV = left ventricular. Asterisks indicate end points of current clinical trials. From *Nat Clin Pract Cardiovasc Med*,[159] with permission.

could conceivably influence cell selection and both the site and method of administration. The evolving pathophysiologic substrates and local microenvironment could also have a crucial effect on cell homing, signaling, survival, and function.

The huge scope of the problem from the bench to the bedside and back again lends itself to the establishment of consortia. The Cardiovascular Cell Therapy Research Network, which comprises 5 institutions and is sponsored by the National Heart, Lung, and Blood Institute, will address a specific series of questions over a 5-year period. Broadly speaking, the major objectives are to develop phase 1 and 2 clinical trials for cell delivery for left ventricular dysfunction (acute MI and chronic heart failure), while defining parameters and models for successful translation of newer cell types[114] (Figure 6).

Clinical Trial End Points

Figure 7[159] illustrates a hierarchical classification of clinical and mechanistic end points, in addition to the overriding objective of safety, which may be used in designing trials. The ultimate proof of efficacy would be a reduction in death and heart failure, and 1 trial of 1400 patients that would be powered to address these end points is in the planning phase (Zeiher A, personal communication, 2009). Nonetheless, for the present, most studies should be mechanistic in focus and, from a practical standpoint, will have to use surrogate end points. Surrogate end points are not ideal, and the literature is replete with evidence showing "that a correlate does not a surrogate make" and that surrogates can be misleading.[160-162] Nonetheless, surrogate end points tailored to the specific question being asked will continue to be the end points of most ongoing trials. These include measurements of global and regional left ventricular function, remodeling, and infarct size, as measured through various imaging modalities. For the end points of myocardial perfusion and metabolism, much needs to be learned about the natural history and variability in controls as well as treated patients. The electrophysiologic "milieu" is amenable to both in vitro and in vivo studies using a variety of techniques. Key evolving issues, such as the genetic, functional, and metabolic function of transplanted stem cells, may lend themselves to the emerging disciplines of biomarkers, metabolomics, proteomics, and molecular imaging.[163] The burgeoning clinical use of left ventricular and biventricular assist devices provides a unique opportunity to use each patient as his or her own control and as a future source of tissue at the time of autopsy or cardiac transplant. The ability to obtain myocardial tissue after stem cell injection offers a window into the effect of different cells on function, morphology, histology, gene and protein expression, and the development of tumors. Nonetheless, the key to the effective use of surrogate end points will be a clear understanding of the mechanistic questions.

Role of Imaging and Stem Cell Tracking

It is likely that sophisticated and evolving imaging technologies will play a valuable if not pivotal role in under-

standing the mechanisms and benefits of cell repair therapy and why cell survival in vivo appears to be so short despite robust evidence of continued growth in vitro. Moreover, by allowing the in vivo tracking of cells, imaging might provide a unique correlate or lack thereof between cell survival and proliferation on the one hand and the functional events on the other.[163] Promising imaging techniques under evaluation include direct labeling with superparamagnetic agents, radioactive tracers (eg, fludeoxyglucose F 18, indium 111, and radioactive indium oxine), and molecular imaging using reporter-gene constructs into stem cells via a viral or nonviral vector.[163-165] In principle, this should enable the study of both engrafted cells and their progeny[164-166]; in this respect, imaging methods and tracers must be able to distinguish viable from nonviable cells.

Safety

Results to date in regard to safety have been encouraging but with the caveats that the number of patients in individual trials is low and both cell retention and survival in the myocardium at 1 week are minimal. In the event that ongoing efforts to increase cell retention, survival, growth, and regenerative potential are successful, potential safety concerns will remain an overriding priority.

Arrhythmogenesis. Whether stem cells are proarrhythmic remains a subject of debate.[167,168] Several earlier studies documented malignant ventricular arrhythmias after skeletal myoblast transplant.[169] Evidence shows that this may be a time-dependent phenomenon with a decline in frequency after 2 to 4 weeks. What remains uncertain is whether the arrhythmias are related to the underlying methods of surgical revascularization and cell delivery[169] and the underlying ischemic substrate.[170] In the largest trial of skeletal muscle myoblasts, an implantable cardioverter-defibrillator was implanted in all patients before discharge, as a precondition for entering the trial; however, in the actual trial the time to first ventricular arrhythmia did not differ between participants receiving placebo and controls.[76] Early animal studies of skeletal myoblasts suggested that transplanted myoblasts remained functionally isolated from the rest of the myocardium.[171] Subsequently, an in vitro coculture model demonstrated sustained reentrant ventricular arrhythmias, probably due to a lack of expression of connexin 43, a protein involved in the formation of gap junctions that are crucial to electromechanical coupling.[172-174]

It remains to be determined whether other cell types will be proarrhythmic. Trials to date have shown no evidence of a proarrhythmic effect, and, if anything, sudden cardiac death may be less frequent in treated groups. Nonetheless, from a conceptual standpoint, the potential for increased arrhythmogenesis exists. Crucial factors may be the efficiency of cell-to-cell coupling, electrical heterogeneity due to incomplete differentiation of cells, the distribution of action potentials at the cell–residual myocardial interface, and gap junction remodeling, in addition to the potentially antiarrhythmic effects of paracrine factors.[22,23,175] It has been postulated that arrhythmia induced by cell injection after MI may be affected by the route of cell delivery and that direct intramyocardial injection may cause mechanical injury and subsequent inflammation that could limit graft survival, cause myocardial damage, and, via the formation of isolated cell clusters, could be arrhythmogenic.[176]

Oncogenic Transformation. Despite abundant experimental evidence of oncogenic transformation, particularly in regard to embryonic and other pluripotent stem cells, no increase in the frequency of tumors has been shown in clinical studies. Nonetheless, the potential exists, and the ability to generate cancer stem cells from normal cells in the experimental situation may provide a unique insight into the process of oncogenic transformation.[174,177] However, recent work has shown that tumorigenic risk is abrogated through guided lineage specification or by selection of early progenitors.[38]

Multiorgan Seeding. That cells injected by the intracoronary route may be identified in the spleen, lungs, and liver is well documented.[159,178] To date, this finding has not been clinically relevant but may become so if the numbers of surviving cells increase markedly.

Aberrant Cell Differentiation. The property of pluripotency implies the potential for cells to differentiate into a variety of cell types. In a mouse model of acute MI, direct intramyocardial injection of unselected bone marrow cells resulted in the induction of substantial intramyocardial calcification[179] and the formation of bone after delivery of MSCs.[180] These data highlight a potential risk of stem cell therapy that awaits further study in humans.

Accelerated Atherosclerosis. One clinical trial showed increased restenosis in patients who underwent stenting soon after MI once granulocyte colony–stimulating factor (GCSF) mobilized bone marrow cells.[181] This finding could be attributed to the inflammatory effects of GCSF administration in the setting of a denuded endothelium. In contrast, 3 meta-analyses of GCSF mobilization of stem cells early after MI have conclusively demonstrated a lack of benefit but also no serious adverse effects on restenosis or other adverse outcomes.[182-184] Other clinical reports have raised the possibility of accelerated atherosclerosis in patients undergoing percutaneous coronary intervention in acute MI.[185] A canine model illustrates the potential for MSCs to cause coronary obstruction and infarction,[186] but whether this will become an issue of clinical importance is uncertain and unlikely.

PHILOSOPHICAL AND ETHICAL ISSUES

Whether stem cell therapy will flourish as part of the therapeutic armamentarium of cardiovascular disease remains in doubt. Clinical studies trend in the right direction, and the extraordinary benefits noted in some animal models augur well for the future. Moreover, the expansion of basic research in the field of regenerative medicine has been massive and global. For these reasons, a sense of cautious optimism seems justified, but at times it would appear that the enthusiasm and allure have moved ahead of the science, and we should heed the lessons from developments in angiogenesis and gene therapy.[187] The first patients were entered into a trial in 1994; after approximately 11 trials during the course of 14 years, no cardiovascular gene product has yet been shown to be clinically effective. The key to future stem cell trials lies in collaboration between scientists and clinicians, academia, the biotechnology industry, and governmental and regulatory agencies, so as to develop the optimal approach to formulating the questions and the specific design of clinical trials. As is the case with all trials, the use of controlled experiments and blinding is pivotal, although in certain clinical scenarios blinding may be difficult and ethically challenging.

The patience of the public and funding agencies is finite and trials are expensive. Health care professionals can play an essential role in the education of the public and the media, so as to dampen unrealistic expectations without losing the support and enthusiasm needed to drive funding. To quote Dr Ann McLaren's commentary in *Cell Stem Cell*: "Of course the public understanding of science is often woefully inadequate, but the scientists' understanding of the public is not much better. Let us aim for an informed dialog, and let us hope that the media will do their best to make sure that nothing is 'lost in translation.'"[188] The dialogue with the media needs to be extended more widely into the public domain, and the relationship between science and society requires informed discussion. A Select Committee from the UK House of Lords in 2000 stated that "Involving the public in decisions about science is like the social equivalent of informed consent."[189] As is usually the case, the rapid development of new science and technology requires a reappraisal of pre-existing paradigms, including some ethical considerations in regard to the changing research environment. The public should be active participants in this discussion.

In general, the fundamental regulatory and ethical requirements that are used in drug and other clinical trials apply equally to cell therapy.[11,190] However, cell therapy trials introduce new ethical issues, including the debate over the use of embryonic material for research; such debates will be influenced by attitudes of governmental administrations, individual state laws, and different approaches to funding within the United States and other countries. The regulatory issues in the European Union surrounding new cardiovascular therapies, and in particular procedures in which cells are manipulated and engineered, are complex and evolving.[191] The lack of precedent in this area of science mandates a close interaction among regulators, scientists, clinicians, and the public because the potential for misunderstanding on all sides is considerable.

Other issues include those of ownership of cell lines, intellectual property, patents, collection of blood in minors (ie, umbilical cord blood donations), and the potential effect of conflict of interest on research study recruitment and analysis of results.[192-194] Because use of patients' autologous stem cells does not directly involve intellectual property, funding of stem cell research by the biotechnology industry has been limited to either cell isolation or delivery devices. Perhaps this situation will change with the emergence of new stem cell lines that are not autologous and the development of unique processing capabilities, but these issues in turn have raised concerns with regard to ownership, price control, and the availability of cell lines. For the present, expensive randomized controlled clinical trials are funded primarily by nonindustrial sources, introducing a difficult challenge for the academic community.[191]

CONCLUSION

The translation of cardiac cell repair therapy into the clinical arena is an intriguing and challenging objective. Whether the future lies in myocyte regeneration or cardiac rejuvenation and protection via cellular, autocrine, and paracrine responses that reduce apoptosis and increase vasculogenesis, inotropism, and metabolic modulation remains to be determined. Perhaps a key to the future of stem cell therapy is an understanding of the genomic and proteomic substrates that modify the multiple signaling and homing systems involved in the transformation of a pluripotent cell into a myocyte. Evidence to date of cell transdifferentiation into myocytes has been disappointing; however, this does not mean that the goal is an unrealistic one. The field is in its relative infancy and the use of different cell types, guided development, and a better understanding of mechanistic concepts may yet bear fruit. Transdifferentiation does occur, albeit at an extremely low frequency, but perhaps this inefficient process can be enhanced by methods directed toward the cell and the substrate. The marriage of gene and cell therapy, as evidenced by the transduction of genes into stem cells so as to modify survival and retention, offers promise, as does the use of stem cells as vectors for drug delivery and as models for understanding the basic biology of disease.

The reality is that the routine clinical use of stem cells for cardiac repair remains an intriguing and tantalizing goal at the end of a long road of exploration. Nonetheless, this fascinating field is making rapid progress, and clinicians should take note and follow with interest, even if from a distance. Eric Hoffer, an American social writer, has stated, "In a time of drastic change, it is the *learners* who inherit the future. The *learned* find themselves equipped to live in a world that no longer exists."[195]

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